PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gary Ruvkun et al.

Art Unit: 1633

Examiner: Sumesh Kaushal, Ph.D.

Serial No.: 09/205,658

Filed: December 3, 1998

Title: THERAPEUTIC AND DIAGNOSTIC TOOLS FOR IMPAIRED GLUCOSE TOLERANCE CONDITIONS

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132 OF GARY RUVKUN, PH.D.

1. I am an inventor on the above-captioned patent application.

2. I have read the Office Action mailed May 27, 1999.

3. The expression of mammalian insulin signaling genes in *C. elegans* does not require the mammalian gene's regulatory elements. It is possible to direct expression of a mammalian gene using regulatory elements of a *C. elegans* gene. For example, in my laboratory, the human insulin gene was expressed under the direction of the *C. elegans* *ins-1* promoter. In addition, mammalian FKHRL1 expression was successfully directed

by the *daf-16* promoter in *C. elegans*. In view of these results it is expected that other members of the mammalian forkhead family, such as FKHR and AFX, can also be expressed in *C. elegans* using, for example, the *daf-16* promoter.

4. It is also possible to express a *C. elegans* gene which is not under the control of its endogenous promoter in *C. elegans*. For example, in my laboratory, we have directed tissue-specific expression of *age-1* in *C. elegans*. *age-1* was expressed in neurons using the pan-neuronal *unc-14* promoter and was expressed in the mechanosensory neurons using the *mec-7* promoter. In addition, *age-1* was expressed in the muscle using the *unc-54* promoter, was expressed in the intestines using a gut-specific *ges-1* synthetic promoter, and was expressed ubiquitously using the *dpy-30* promoter. Each of these promoter-*age-1* fusion constructs produced AGE-1 protein which was capable of rescuing dauer arrest mediated by a *C. elegans age-1* (*mg44*) mutant. In sum, these results demonstrate that it is not necessary to use the endogenous regulatory regions of a mammalian or nematode *daf* gene, such as *daf-18* or PTEN, to direct expression of a DAF protein.

5. *Daf* genes are believed to play a role in the regulation of metabolism through TGF-β and insulin signaling pathways. *C. elegans* members of the TGF-β signaling pathway include DAF-7, DAF-4, DAF-1, DAF-8, DAF-14, and DAF-3. Mammalian members of many of these protein families have also been identified. For example, DAF-7 is a particular subtype of the TGF-β superfamily. In addition, DAF-1 and DAF-4 are *C.*

elegans members of the type I and type II TGF-β receptor family, respectively; and DAF-3, DAF-8, and DAF-14 are *C. elegans* members of the Smad family of proteins.

6. Mammalian gene products thought to be involved in the insulin signaling pathway include insulin, the insulin receptor, PI-3 kinase, PDK1 kinase, AKT/PKB kinase, and the particular forkhead proteins FKHR, FKHRL1, and AFX. *C. elegans* relatives of many of these mammalian gene products have been identified. For example, DAF-2 is the *C. elegans* ortholog of the human insulin receptor superfamily; AGE-1 is the *C. elegans* ortholog of human PI-3 kinase; PDK-1 is the *C. elegans* ortholog of human PDK1; *C. elegans* AKT-1 and AKT-2 are orthologs of human AKT kinase; and DAF-16 is the *C. elegans* ortholog of human FKHR, FKHRL1, and AFX.

7. There is a clear association between *daf* genes and the onset of impaired glucose tolerance conditions associated with obesity. For example, *C. elegans daf-2* mutants are normally dauer-arrested and exhibit increased fat accumulation when compared to their wild-type counterparts. When the *daf-2* mutants are rescued from dauer arrest (for example, by mutation of the *daf-16* or *daf-18* gene) and therefore from an impaired glucose tolerance condition, they also exhibit lower fat accumulation levels. Similar results are observed in *C. elegans age-1* mutants. These mutants also exhibit increased fat accumulation as dauers, and, upon rescue from dauer arrest by expression of the wild-type AGE-1 protein, similarly exhibit lower fat accumulation levels. These experimental results support Applicants' position that *daf* genes are involved in impaired

glucose tolerance and obesity.

8. *C. elegans* in which the *cod-5/tph-1* gene, encoding the serotonin synthesis protein, tryptophan hydroxylase, is knocked out make no serotonin. Such *cod-5/tph-1* mutant animals exhibit phenotypes which are characteristic of *daf* mutants: up to half of the *cod-5/tph-1* mutant animals arrest at the dauer stage and accumulate large amounts of fat. In addition, there is a lower expression of DAF-7 in *cod-5/tph-1* mutants. These similarities indicate that there is a serotonin input to the *daf-7* pathway. Serotonin also acts upstream of the insulin signaling pathway. For example, a *daf-16* mutation suppresses the dauer arrest and increase in fat accumulation of *cod-5/tph-1* mutants, again providing evidence that the presently claimed screens are useful for identifying compounds involved in obesity. It is well known that the serotonergic pathway is a target of therapeutics used to treat obesity, and the involvement of serotonin in the *daf* pathway indicates that *daf*-related screens would similarly be useful for isolating anti-obesity therapeutics in our claimed system.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 10/26/99

Gary Ruvkun, Ph.D.